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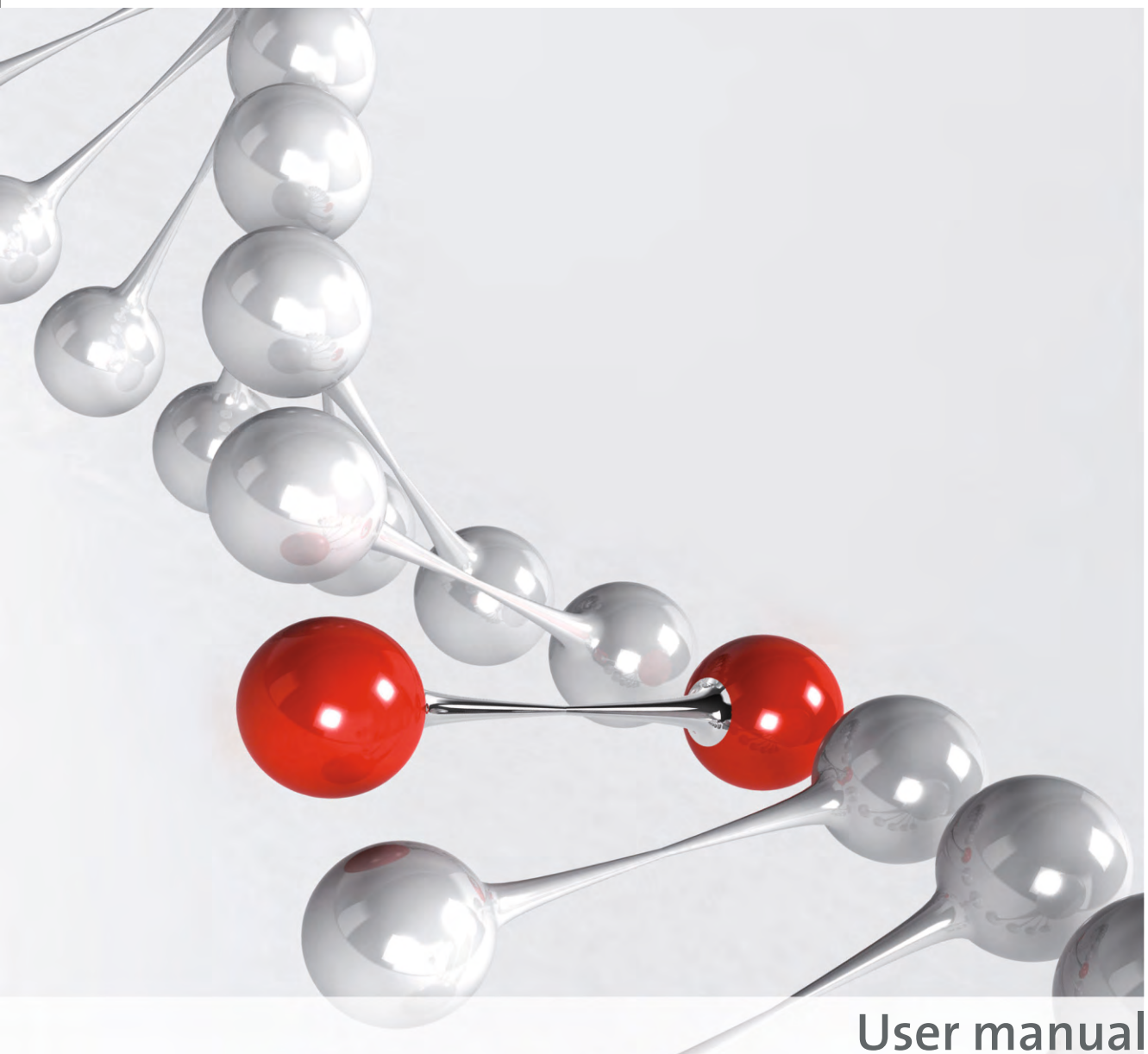

User manual
InviMag® SalivaGene DNA Kit/ KF96

for use on KingFisher® 96 and KingFisher® Flex, Thermo Fisher Scientific

for automated purification of total DNA from SalivaGene stabilized clinical swab (mouth brushes) & saliva samples with magnetic beads

REF 7435060X00 STRATEC Molecular GmbH, D-13125 Berlin

Instruction for InviMag® SalivaGene DNA Kit/ KF96

The **InviMag® SalivaGene DNA Kit /KF96** is used to stabilize and purify human and/or bacterial genomic DNA from salivagene quantitatively by use of magnetic beads.
The **InviMag® SalivaGene DNA Kit /KF96** is a modular system using a stabilization solution for collection, stabilization, storage and transportation of saliva material without degradation of DNA for at least 12 months with a very efficient isolation method for high quality DNA.

The kit is neither suitable for the isolation of DNA from stool samples or fungi nor for purification of total RNA.

Trademarks: InviMag®, SalivaGene®, Invisorb®, PSP®. Registered marks, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

The Invisorb® technology is covered by patents and patent applications: US 6,110363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

InviMag®, SalivaGene®, Invisorb® and PSP® are registered trademarks of STRATEC Biomedical AG.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

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Kit content of the InviMag® SalivaGene DNA Kit/ KF96

Store lyophilized Lysozyme at 2–8°C! Store dissolved Proteinase K and Lysozyme at –20°C! Store all other kit components at room temperature (RT)!

	1x 96 extractions	5x 96 extractions
Catalogue No.	7435060100	7435060200
Lysozyme	for 2 x 1.1 ml working solution	for 10.5 ml working solution
Binding Buffer A	9 ml (final volume 30 ml)	36 ml (final volume 120 ml)
Proteinase K	for 2 x 1.1 ml working solution	for 10.5 ml working solution
MAP Solution B	2 x 1.1 ml	10.5 ml
Wash Buffer I	30 ml (working solution 60 ml)	2 x 80 ml (2 x 160 ml working solution)
Wash Buffer II	45 ml (working solution 150 ml)	3 x 60 ml (3 x 200 ml working solution)
Elution Buffer	15 ml	60 ml
2.0 ml Deep Well Plate	4	20
KF 96 Tip Comb for DW magnets	1	5
200 µl Elution Plate*	2	2 x 5
Sealing Foils	2	10
Manual	1	1
Initial steps	Add 21 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min.. Shortly before use mix by inverting several times. Add 1.1 ml ddH_2O to each tube with **Proteinase K,** mix thoroughly until completely dissolving and store the tubes at -20°C! Add 1.1 ml ddH_2O to each tube with **Lysozyme,** mix thoroughly and store the tubes at -20°C! Add 30 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and store with tightly closed cap at RT. Add 105 ml of 96-100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and store with tightly closed cap at RT.	Add 84 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min.. Shortly before use mix by inverting several times. Add 10.5 ml ddH_2O to the bottle with **Proteinase K,** mix thoroughly until completely dissolving and store the tubes at -20°C! We recommend to make aliquots and store them at –20°C. Add 10.5 ml ddH_2O to the bottle with **Lysozyme,** mix thoroughly and store the tubes at -20°C! We recommend to make aliquots and store them at –20°C. Add 80 ml of 96-100% ethanol to each bottle **Wash Buffer I**, mix thoroughly and store with tightly closed cap at RT. Add 140 ml of 96-100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and store with tightly closed cap at RT.

Kit content of the InviMag® SalivaGene DNA Kit/ KF96 w/o plastic

Store lyophilized Lysozyme at 2–8°C! Store dissolved Proteinase K and Lysozyme at –20°C! Store all other kit components at room temperature (RT)!

	1x 96 extractions	5x 96 extractions
Catalogue No.	7435060150	7435060250
Lysozyme	for 2 x 1.1 ml working solution	for 10.5 ml working solution
Binding Buffer A	9 ml (final volume 30 ml)	36 ml (final volume 120 ml)
Proteinase K	for 2 x 1.1 ml working solution	for 10.5 ml working solution
MAP Solution B	2 x 1.1 ml	10.5 ml
Wash Buffer I	30 ml (working solution 60 ml)	2 x 80 ml (2 x 160 ml working solution)
Wash Buffer II	45 ml (working solution 150 ml)	3 x 60 ml (3 x 200 ml working solution)
Elution Buffer	15 ml	60 ml
Sealing Foils	2	10
Manual	1	1
Initial steps	Add 21 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min.. Shortly before use mix by inverting several times. Add 1.1 ml ddH_2O to each tube with **Proteinase K,** mix thoroughly until completely dissolving and store the tubes at -20°C! Add 1,1 ml ddH_2O to each tube with **Lysozyme,** mix thoroughly and store the tubes at -20°C! Add 30 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and store with tightly closed cap at RT. Add 105 ml of 96-100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and store with tightly closed cap at RT.	Add 84 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min.. Shortly before use mix by inverting several times. Add 10.5 ml ddH_2O to the bottle with **Proteinase K,** mix thoroughly until completely dissolving and store the tubes at -20°C! We recommend to make aliquots and store them at –20°C. Add 10.5 ml ddH_2O to the bottle with **Lysozyme,** mix thoroughly and store the tubes at -20°C! We recommend to make aliquots and store them at –20°C. Add 80 ml of 96-100% ethanol to each bottle **Wash Buffer I**, mix thoroughly and store with tightly closed cap at RT. Add 140 ml of 96-100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and store with tightly closed cap at RT.
Plastic to be supplied by user (see order information)		
KF 96 Tip Comb for DW magnets	1	5
200 µl Elution Plate*	2	2 x 5
2.0 ml Deep Well Plate	4	20

Content of SalivaGene® Stabilization Sets

Collection Sets	50 sets
SalivaGene® Collection Module II	1035210800
DNA Stabilizer (2 ml SalivaGene® DNA Stabilizer) prefilled in a Transport Tube	10 x 50 container
SalivaGene® Collection Tube, provided as Set	10 x 50 container
SalivaGene® Swab Collection Tube prefilled with DNA Stabilizer (500 µl SalivaGene DNA Stabilizer)	10 x 50 pieces
SalivaGene® Buccal Swab	1035230300
Buccal Swabs	10 x 50 pieces
SalivaGene® Swab Collection Tube prefilled with DNA Stabilizer (500 µl SalivaGene DNA Stabilizer)	10 x 50 pieces

Symbols

Lot number

Catalog number

Expiry date

Consult operating instructions

Temperature limitation

Do not reuse

Manufacturer

Storage

All buffers and kit components, except the dissolved **Proteinase K** and the **Lysozyme** of the **InviMag® SalivaGene DNA Kit/ KF96** should be stored well sealed and dry at room temperature and are stable for at least 12 months under these conditions. **Room temperature (RT) is defined as range from 15-30°C.**

Dissolved **Proteinase K** and **Lysozyme** must be stored at -20 °C. Repeated freezing and thawing will reduce the activity of **Proteinase K** and **Lysozyme** dramatically. Dividing of **Proteinase K** and **Lysozyme** into aliquots and storage at -20°C is recommended.

Wash Buffers and **Binding Buffer** charged with either ethanol or isopropanol should be appropriate sealed and stored at RT.

The **MAP Solution B** (magnetic beads) should be stored at 2-8°C
Before use make sure that all components have room temperature. If there are any precipitates visible within the provided solutions dissolve them by carefully warming up to 30°C.

Quality control and product warranty

STRATEC Molecular warrants the correct function of the **InviMag® SalivaGene DNA Kit/ KF96** for applications as described in this manual. Purchaser must determine the suitability of the product for its particular use. Should any product fail to perform the applications as described in

the manual, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a problem in the lot, STRATEC Molecular will replace the product free of charge.

STRATEC Molecular reserves the right to change, alter, or modify any product to enhance its performance and design at any time.

In accordance with STRATEC Molecular's ISO 9001-2000 and ISO EN 13485 certified Quality Management System the performance of all components of the **InviMag® SalivaGene DNA Kit/ KF96** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

If you have any questions or problems regarding any aspects of **InviMag® SalivaGene DNA Kit/ KF96** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

For technical support or further information please contact:

from Germany +49-(0)30-9489-2901/ 2910
from abroad +49-(0)30-9489-2907 or contact your local distributor.

Intended use

The **InviMag® SalivaGene DNA Kit/ KF96** is suitable for a reproducible purification of total DNA from up to 500 µl stabilization media of saliva or swab samples in forensic, human-identity, genetic, biosecurity and pathogen analyses. The purification is very efficient and the isolated DNA performs well in downstream applications, such as quantitative PCR and STR analysis, with high signal-to-noise ratios.

The system consists of two modules, one collection set and one extraction module, useable independently or in combination.

THE PRODUCT IS INDENTED FOR USE BY PROFESSIONALS SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of DNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

Product use limitation

The kit is neither validated for the isolation of genomic DNA from serum, plasma, fungi or from parasites nor for isolation and purification of total RNA.

The included chemicals are only useable once.

Differing of starting material or flow trace may lead to inoperability; therefore neither a warranty nor guarantee in this case will be given, neither implied nor express.

The user is responsible to validate the performance of the STRATEC Molecular product for any particular use. STRATEC Molecular does not provide for validation of performance characteristics of the product with respect to specific applications. STRATEC Molecular products may be used e.g.in clinical diagnostic laboratory systems conditioned upon the complete diagnostic system of the laboratory the laboratory has been validated pursuant to CLIA' 88 regulations in the U.S. or equivalents in other countries.

All products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.

The chemicals and the plastic parts are for laboratory use only; they must be stored in the laboratory and must not be used for purposes other than intended.

The product and its content are unfit for consumption.

Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!

Avoid skin contact! Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at **www.stratec.com** for each STRATEC Molecular product and its components. If buffer bottles are damaged or leaking, **WEAR GLOVES, AND PROTECTIVE GOGGLES** when discarding the bottles in order to avoid any injuries.

STRATEC Molecular has not tested the liquid waste generated by the **InviMag® SalivaGene DNA Kit/ KF96** procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered infectious and be handled and discarded accordingly to local safety regulations.

European Community risks and safety phrases for the components of the **InviMag® SalivaGene DNA Kit/ KF96** to which they apply are listed below as follows:.

Wash Buffer I

warning
H302-312-332-412 EUH032 P273

Proteinase K:

danger
H315-319-334-335 P280-305-351-338-310-405

SalivaGene® DNA Stabilizer

danger
H319 P305-351-338

H319:	Causes serious eye irritation.
H302:	Harmful if swallowed.
H312:	Harmful in contact with skin.
H332:	Harmful if inhaled.
H412:	Harmful to aquatic life with long lasting effects.
EUH032:	Contact with acids liberates very toxic gas.
H315:	Causes skin irritation.
H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335:	May cause respiratory irritation.
P305+P351+P338:	If in eyes: Rinse cautiously with water for several minutes. If present, remove contact lenses and continue rinsing.
P273:	Avoid release to the environment.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P310:	Immediately call a POISON CENTER or doctor/physician.
P405:	Store locked up.

Emergency medical information can be obtained 24 hours a day from infotrac:

outside of USA: **1 – 352 – 323 – 3500**

inside of USA : **1 – 800 – 535 – 5053**

Product characteristic of the InviMag® SalivaGene DNA Kit/ KF96

Starting material	Yield	Time	Ratio
500 µl of stabilization buffer (SalivaGene DNA Stabilizer) saliva mix 1:1 or 500 µl of swab stabilization (SalivaGene DNA Stabilizer)	up to 10 µg (depends on starting material)	40 - 50 min (depends on the used protocol)	A_{260} : A_{280} 1.7 – 2.0

The **InviMag® SalivaGene DNA Kit/ KF96** uses the **PSP® Technology** (Preanalytical-Sample-Processing) a patented cost efficient technology and is a modular designed system for collection, stabilization, storage and transportation of saliva samples with DNA purification. The system combines the use of a prefilled **SalivaGene® Collection Tube** for saliva or swab sample collection, the storage and stabilization of saliva specimen without any degradation of the DNA during transportation, including the prelysis of bacteria and a very efficient and fast isolation (about 40-50 min) of high quality total DNA from up to 500 µl sample mixture. The kit is designed for isolation of DNA from host organism as well as for isolation of DNA from pathogen microorganisms.

After collection, the sample is transferred to the **SalivaGene® Collection Tube** prefilled with **SalivaGene® DNA Stabilizer.** The **SalivaGene® DNA Stabilizer** leads to an inactivation of DNases and prevents the degradation of the DNA, preserves the microorganism titer and prelyses bacteria. The transport of the stabilized DNA can be carried out in the **SalivaGene® DNA Stabilizer** containing tubes without cooling for at least 12 months at ambient temperature. The sample can alternatively be stored in this buffer at -20°C for long-term storage. For the DNA extraction process an aliquot or the whole volume is used. The residual sample can be stored and used for further extractions.

A 500 µl aliquot of the stabilized sample is used in a **PSP® SalivaGene DNA** isolation procedure. At first, the proteins are degraded with Proteinase K, followed by binding of the DNA to magnetic beads while contaminants, like potent inhibitors and proteins, are rejected and removed leaving the pure DNA to be eluted in Elution Buffer. In case of extracting bacterial DNA an additional lysis step with Lysozyme is performed.

For reproducible and high yields, appropriate sample storage is essential. Yields may be varying from sample to sample depending on factors such as the health of the donor, patient medication, or sample storage conditions.

The procedure neither requires a phenol / chloroform extraction nor an alcohol precipitation. The method requires only minimal user interaction allowing safe handling of potentially infectious samples. The procedure is designed to avoid sample-to-sample cross-contamination.

Due to the high purity, the isolated genomic DNA is ready to use for a broad panel of downstream applications (see below) or can be stored at –20°C for subsequent use.

- PCR*, real-time PCR*, RFLP / AFLP*- analysis
- restriction enzyme digestion
- HLA-typing
- pathogen detection

*) The PCR method is covered by U.S. Patents 4,683,195 and 4,683,202 owned by Hoffmann-LaRoche Inc. The purchase of the **PSP® SalivaGene DNA Kit** cannot be construed as an authorization or implicit license to practice PCR under any patents held by Hoffmann-LaRoche Inc.

Sampling and sample storage

Starting material

The amount of starting material used in the **InviMag® SalivaGene DNA Kit/ KF96** procedure can vary slightly, depending on the amount of DNA present in the sample. Specific guidance for starting amounts is given in the individual protocols.
All protocols work with aliquots of the transport media, in which fresh saliva samples are collected. The DNA is stable for up to 12 months at room temperature in the transport media (**SalivaGene® DNA Stabilizer**). The aliquots of the stabilization buffer may be processed on the same day as collection or stored for future processing. For long-term storage freeze the sample at -20°C.

STRATEC Molecular will be released of its responsibilities if other sample materials than described in the Intended Use are processed or if the sample preparation protocols are changed or modified.

Principle and procedure

The **InviMag® SalivaGene DNA Kit/ KF96** procedure comprises following steps:

- collection of the material using a **SalivaGene® Collection Set**
- lysis of cells in presence of non-chaotropic conditions at elevated temperature in the Stabilization Buffer (**SalivaGene® DNA Stabilizer**)
- protein digestion (**Proteinase K**)
- binding of the genomic DNA to magnetic beads (**MAP Solution B**)
- washing of the beads and elimination of contaminants and ethanol
- elution of pure genomic or bacterial DNA

This manual contains two protocols, according to the different requirements of the starting materials and the extraction instrument used.

Lysis
Samples are lysed in presence of non-chaotropic conditions at elevated temperature in the Stabilization Buffer (**SalivaGene® DNA Stabilizer**).

Protein digestion
Proteins are digested in the lysed samples in the presence of **Proteinase K**.

Binding genomic DNA
By adding **Binding Buffer A** to the lysate, optimal binding conditions are adjusted.
Each lysate is then mixed with magnetic beads and the genomic DNA is bound to the beads.

Removing residual contaminations
Contaminants are efficiently removed using **Wash Buffer I and II**, while the genomic, mitochondrial or bacterial DNA remains bound to the magnetic beads.

Elution of pure genomic DNA
The nucleic acids are eluted from the magnetic beads using **Elution Buffer** and are ready-to-use in different downstream applications.

Yield and quality of DNA

The amount of purified DNA in the **InviMag® SalivaGene DNA Kit/ KF96** procedure from different samples depends on the sample type, sample source, transport, storage and age. Yield and quality of isolated DNA are suitable for any downstream detection system. The diagnostic tests should be performed accordingly to manufacturer's specifications.

Important points before starting a protocol

General handling notes

Immediately upon receipt of the product, inspect the product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities you have to notify STRATEC Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to "Safety Information" (see page 7). Do not use damaged kit components because their use may lead to poor kit performance.

- Always change pipette tips between different liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipette tips.
- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
- Discard contaminated gloves immediately.
- Do not combine components of different kits, unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar airflow until the samples are lysed.
- This kit should only be used by trained personnel.

Preparing reagents and buffers

Before starting a run, equilibrate all reagents at room temperature. Where necessary, gently mix and redissolve any precipitates by warming up to 30°C. Swirl gently to avoid foaming.

1. Add the required amount of ddH_2O to **Proteinase K** containing tube (see below). Vortex for 5 s. Repeat this step for the **Lysozyme.**
2. Add the required amount of ethanol to **Wash Buffer I** and **Wash Buffer II** and isopropanol to **Binding Buffer A**
3. **Elution Buffer** is ready-to-use.

1x 96 DNA extractions:
Add 21 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.
Add 1.1 ml ddH_2O to each tube with **Proteinase K,** mix thoroughly until completely dissolving and store the tubes at -20°C!
Add 1.1 ml ddH_2O to each tube with **Lysozyme,** mix thoroughly and store the tubes at -20°C!
Add 30 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and store with tightly closed cap at RT.
Add 105 ml of 96-100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and store with tightly closed cap at RT.
5x 96 DNA extractions:
Add 84 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.
Add 10.5 ml ddH_2O to the bottle with **Proteinase K,** mix thoroughly until completely dissolving and store the tubes at -20°C! We recommend making aliquots and storing them at –20°C.
Add 10.5 ml ddH_2O to the bottle with **Lysozyme,** mix thoroughly and store the tubes at -20°C! We recommend making aliquots and storing them at –20°C.
Add 80 ml of 96-100% ethanol to each bottle **Wash Buffer I**, mix thoroughly and store with tightly closed cap at RT.
Add 140 ml of 96-100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and store with tightly closed cap at RT.

Reagents and equipment to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at **www.stratec.com** under each STRATEC Molecular kit and kit component.

- Measuring cylinder (250 ml)
- Disposable gloves
- Pipette and pipette tips
- Vortexer
- ddH_2O
- 96-100% ethanol
- Isopropanol*

*The **InviMag® SalivaGene DNA Kit/ KF96** is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from **Carl Roth**

*** Possible suppliers for Isopropanol:**

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

Sigma
2-Propanol
Order no. 59304-1L-F

Important indications

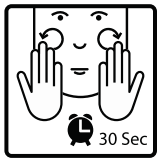
- Samples and buffers should be thoroughly mixed and should have room temperature.
- The elution can be done by using lower amount of **Elution Buffer**. This may result in a higher concentration of DNA but pay attention that minimum volume for elution is 50 µl. However, this will lead to a reduced yield and we strongly recommend to never use a volume smaller than 100 µl.
- The volume of the eluted DNA may be lower than the added volume of **Elution Buffer**.
- The **Elution Buffer** doesn't contain EDTA.
- The yield can be increased, if the incubation time with **Elution Buffer** will be prolonged.

PSP®-Treatment with SalivaGene® Collection Module II

Intended Use: Collection of human saliva samples for DNA stabilization and extraction (using the SalivaGene DNA Extraction Kits).
The DNA in SalivaGene stabilized samples is stable for 1 year at room temperature within the expiry date. The product is intended for use under supervision of professionals only, such as technicians, physicians and biologist trained in molecular biological techniques.

Contents: Collection Tube, Stabilizer Tube. The Stabilizer Tube contains approx. 2 ml of liquid Saliva DNA Stabilizer reagent.

Limitations of the procedure: Do not eat, drink, smoke or chew gum at least 30 min prior to saliva collection. Collecting 2 ml of saliva may require several minutes. Putting some grains of sugar on the tongue helps to produce saliva and does not interfere with the stabilization.

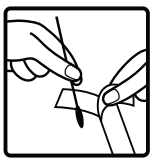
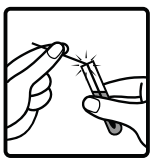
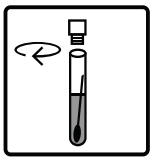
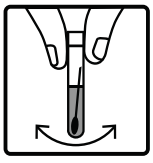
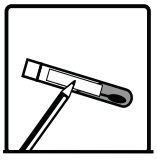
1.	Unscrew lid from Collection Tube and put it aside for further use.
2. 	Rub cheeks against teeth intensely for 30 sec.
3.	Collect saliva to indicated fill level, avoid making and measuring air bubbles.
4.	Unscrew and discard lid from Stabilizer Tube. Pour Saliva DNA Stabilizer reagent into Collection Tube.
5.	Screw lid tightly onto the Collection Tube again.
6.	Shake tube for 15 sec to mix saliva and Saliva DNA Stabilizer reagent.

PSP-Treatment of SalivaGene® Buccal Swab

Intended Use: Collection of human oral samples for DNA stabilization and extraction (using the SalivaGene DNA Extraction Kits).
The DNA in SalivaGene stabilized samples is stable for 1 year at room temperature within the expiry date. The product is intended for use under supervision of professionals only, such as technicians, physicians and biologist trained in molecular biological techniques.

Contents: Swab, Stabilizer Tube, label for donor description. The Stabilizer Tube contains approx. 2 ml of liquid DNA Stabilizer reagent.

Limitations of the procedure: Do not eat, drink, smoke or chew gum at least 30 min prior to swab collection. Ensure the swab tip does not come into contact with any surface prior to collection. Put the Stabilizer tube upright to prevent the liquid inside the tube from spilling. Be sure to move the swab over the entire cheek and to moisten it with saliva.

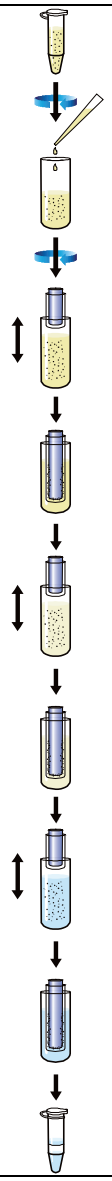
1. 	Open swab package and remove swab without touching the tip.
2.	Insert swab into the mouth and rub swab tip firmly against the inner cheeks for about 30 seconds on each side.
3.	Unscrew lid from Stabilizer Tube and put it aside for further use. Insert swab into the Stabilizer Tube.
4. 	Break swab tip at breaking point. Discard the broken handle part.
5. 	Screw lid tightly onto the Stabilizer Tube again.
6. 	Gently shake tube for 15 sec to mix buccal cells and DNA Stabilizer reagent.
7. 	Label Stabilizer Tube with donor name and collection date using provided label.

Scheme of the InviMag® SalivaGene DNA Kit /KF96

Please read protocols prior the start of the preparation carefully

Important: Prefill all plates with the appropriate buffers and volumes as described below before starting a run.

Tip Plate:	Insert the KF96 Tip Comb for DW magnets on a Tip Plate*
Binding Plate:	Prepare the Binding plate as described below
Washing Plate_1:	Add **500 µl Wash Buffer I** to a 2.0 ml Deep Well Plate
Washing Plate_2:	Add **600 µl Wash Buffer II** to a 2.0 ml Deep Well Plate
Washing Plate_3:	Add **600 µl Wash Buffer II** to a 2.0 ml Deep Well Plate
Elution Plate:	Add **150 µl Elution Buffer** to a KF Elution Plate (same size as Tip Plate)



Bacteria DNA: Transfer 500 µl stabilized saliva into the Lysis Plate, add 20 µl of Lysozyme and start the program "InviMag® SalivaGene DNA Bacteria KFflex96" or "InviMag® SalivaGene DNA Bacteria KF96". After the first lysis step the user will be prompted to add 20 µl Proteinase K and confirm it by pressing start.

Human DNA:
Transfer 500 µl of the stabilized sample into the Lysis Plate, add 20 µl Proteinase K and start the program "InviMag® SalivaGene DNA Human KFflex96"/ "InviMag® SalivaGene DNA Human KF96".

After the lysis step, the user will be prompted to add 200 µl **Binding Buffer A** *(follow preparing instructions)* and 20 µl **MAP Solution B** during a pause.

DNA binds to magnetic particles

Magnetic separation

Washing of the particle fixed genomic DNA

Magnetic separation

Elution of nucleic acids

Magnetic Separation

Pure DNA

* Elution Plates and Tip Plates are identically. Use one provided Elution Plate as a Tip Plate.

Protocol 1: Human genomic DNA extraction from stabilized material

Please read the instructions carefully and conduct the prepared procedure.

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer A*** *– see instruction page: 10*

Important: *For working with the KingFisher instrument, please carefully read the manufacturer's documents !*

1. Switch on the KF96 / KFflex96 instrument
2. Prefill all Deep Well Plates as described below.

Note*: Please avoid evaporation of the prefilled buffer components by sealing the Deep Well Plates with a sealing foil or with parafilm!*

Important: *Mix the bottle with the* ***MAP B Solution*** *by vigorously vortexing!*

Binding Plate:	Carefully transfer 500 µl of the stabilized sample into the cavity of the Binding Plate (2 ml DWP) prefilled with 20 µl **Proteinase K**.
Washing plate_1:	Add 500 µl **Wash Buffer I** into the cavities of a 2 ml Deep Well Plate
Washing plate_2:	Add 600 µl **Wash Buffer II** into the cavities of a 2 ml Deep Well Plate
Washing plate_3:	Add 600 µl **Wash Buffer II** into the cavities of a 2 ml Deep Well Plate
Elution Plate:	Add 150 µl **Elution Buffer** into the cavities of the KF Elution Plate

3. Choose the program **"InviMag® SalivaGene KFflex96_Human"** or **"InviMag® SalivaGene KF96_Human"** and press the "START" button.
4. Load the prefilled plates into the KingFisher instrument by following the specification given on the display and confirm every step by pressing the button "START".

When all prefilled plates are loaded into the KingFisher machine press the button "START" again to execute the assay file. The run will last approximately 40-50 minutes.

After protein degradation, the machine will be paused and 20 µl **MAP Solution B** and 200 µl **Binding Buffer A** have to be added to each sample containing cavity in the Binding Plate. After addition of both reagents, press the "START" button once again to continue with the run. From here, the machine will finish the purification process without any further user interaction.

Protocol 2: Bacterial DNA extraction from stabilized material

Please read the instructions carefully and conduct the prepared procedure.

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer A*** *– see instruction, page 10*

Important: *For working with the KingFisher instrument, please carefully read the manufacturer´s manual!*

5. Switch on the KF96 / KFflex96 instrument

6. Prefill all Deep Well Plates as described below.

Note*: Please avoid evaporation of the prefilled buffer components by sealing the Deep Well Plates with a sealing foil or with parafilm!*

Important: *Mix the bottle with the* ***MAP B Solution*** *by vigorously vortexing!*

Binding Plate:	Carefully transfer 500 µl of the sample into the cavity of the Binding Plate (2 ml DWP) prefilled with 20 µl **Lysozyme**.
Washing plate_1:	Add 500 µl **Wash Buffer I** to a 2 ml Deep Well Plate
Washing plate_2:	Add 600 µl **Wash Buffer II** to a 2 ml Deep Well Plate
Washing plate_3:	Add 600 µl **Wash Buffer II** to a 2 ml Deep Well Plate
Elution Plate:	Add 150 µl **Elution Buffer** to a KF Elution Plate

7. Choose the program **"InviMag SalivaGene KFflex96_Bacteria"** or **"InviMag SalivaGene KF96_Bacteria"** on the display of the KingFisher instrument and press the "START" button.

8. Load the prefilled plates into the KingFisher instrument by following the specification given on the display and confirm every following step by pressing the "START" button.

9. When all prefilled plates are loaded into the KingFisher machine press the "START" button again to execute the assay. The run will last about 50 minutes.

After the first lysis step, a pause occurs and 20 µl **Proteinase K** has to be added to each sample containing cavities of the Binding Plate. After Proteinase K distribution press the "START" button to continue with the lysis.

After the second lysis step a second pause occurs and 200 µl **Binding Buffer A** and 20 µl **MAP Solution B** have to be added to sample containing cavities of the Binding Plate. After adding both reagents, press the "START" button again to continue with the assay. From this point, the instrument will continue without any further user interaction.

For self-programming of the KF96 and KFflex96 instrument (Bacteria protocol)

Reagent info

Lysis Plate		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Salivagene Sample	500	-	Sample

Washing Plate 1		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer I	500	-	Reagent

Washing Plate 2		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer II	600	-	Reagent

Washing Plate 3		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer II	600	-	Reagent

Elution Plate		KingFisher 96 KF plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Elution Buffer	150	-	Reagent

Tip Plate		KingFisher 96 KF plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
-	-	-	-

Dispensed reagents

Lysis Plate		Microtiter DW 96 plate	
Name	**Step**	**Well volume [µl]**	**Total reagent volume [µl]**
Proteinase K	Add_ProteinaseK	20	-
Binding Buffer + MAP B Solution	Adjust Binding	220	-

Steps data

Tip		96 DW tip comb	
	Pick-Up	Tip Plate	
	Lysis1	Lysis Plate	
	Beginning of step	Precollect	No
		Release beads	No
	Mixing / heating:	Mixing time, speed	00:10:00, Medium
		Heating temperature [°C]	37
		Preheat	Yes
	End of step	Postmix	No
		Collect beads	No
	Add_ProteinaseK	Lysis Plate	
		Message	Add Proteinase K
		Dispensing volume [µl]	20
	Reagent(s)	Name	Proteinase K
		Volume [µl]	20
	Lysis2	Lysis Plate	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:20:00, Medium
		Heating temperature [°C]	60
		Preheat	Yes
	End of step	Postmix	No
		Collect beads	No
	Adjust Binding	Lysis Plate	
		Message	Add Beads + Buffer
		Dispensing volume [µl]	220
	Reagent(s)	Name	Binding Buffer + MAP B Solution
		Volume [µl]	220
	Binding	Lysis Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:05:00, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	10

	Wash1	Washing Plate 1	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:30, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	7
	Wash2	Washing Plate 2	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	7
	Wash3	Washing Plate 3	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	7
	Dry	Washing Plate 3	
		Dry time	00:03:30
		Tip position	Outside well / tube
	Elution	Elution Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Medium
	Mixing / heating:	Mixing time, speed	00:10:00, Slow
		Heating temperature [°C]	60
		Preheat	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	10
	Bead Removal	Washing Plate 3	
		Release time, speed	00:00:30, Fast
	Leave	Tip Plate	

For self-programming of the KF96 and KFflex96 instrument (Human protocol)

Reagent info

Lysis Plate			Microtiter DW 96 plate
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Salivagene Sample	500	-	Reagent
Proteinase K	20	-	Reagent

Washing Plate 1			Microtiter DW 96 plate
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer I	500	-	Reagent

Washing Plate 2			Microtiter DW 96 plate
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer II	600	-	Reagent

Washing Plate 3			Microtiter DW 96 plate
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer II	600	-	Reagent

Elution Plate			KingFisher 96 KF plate
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Elution Buffer	150	-	Reagent

Tip Plate			Microtiter DW 96 plate
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
-	-	-	-

Dispensed reagents

Lysis Plate			Microtiter DW 96 plate
Name	**Step**	**Well volume [µl]**	**Total reagent volume [µl]**
Binding Buffer + MAP B Solution	Adjust Binding	220	-

Steps data

Tip		96 DW tip comb	
	Pick-Up	Tip Plate	
	Lysis	Lysis Plate	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:20:00, Medium
		Heating temperature [°C]	60
		Preheat	Yes
	End of step	Postmix	No
		Collect beads	No
	Adjust Binding	Lysis Plate	
		Message	Add Beads + Buffer
		Dispensing volume [µl]	220
	Reagent(s)	Name	Binding Buffer + MAP B Solution
		Volume [µl]	220
	Binding	Lysis Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:05:00, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	10
	Wash1	Washing Plate 1	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:30, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	7
	Wash2	Washing Plate 2	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	7

	Wash3	Washing Plate 3	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	7
	Drying	Washing Plate 3	
		Dry time	00:03:30
		Tip position	Outside well / tube
	Elution	Elution Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Medium
	Mixing / heating:	Mixing time, speed	00:10:00, Slow
		Heating temperature [°C]	60
		Preheat	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	10
	Bead Removal	Washing Plate 3	
		Release time, speed	00:00:30, Fast
	Leave	Tip Plate	

Troubleshooting

Problem	Cause	Comments and suggestions
low amount of DNA	insufficient cell lysis due to decreased **Proteinase K** activity	repeat the DNA purification procedure with a new sample and freshly prepared **Proteinase K** stock solution. Be sure to store the stock solution at -20°C
	insufficient lysis due to insufficient mixing with lysis buffer **- SalivaGene® DNA Stabilizer**	repeat the DNA purification procedure with a new sample. Be sure to mix the sample and **SalivaGene® DNA Stabilizer** immediately elongation of the stabilization/ lysis procedure to overnight significantly increases the overall yield
	low percentage alcohol used	repeat purification procedure with a new sample and/or prepare a new bottle of Wash Buffer if a wrong concentration of ethanol was used
	incomplete elution	increase incubation time with **Elution Buffer** to 5-10 min or increase the elution volume to 200 µl this will however reduce the final DNA concentration
	pH of water incorrect (acidic)	low pH may reduce DNA yield; ensure that the pH of the water is at least 7.0 or use **Elution Buffer** (contains 10 mM Tris-HCl but no EDTA)
degraded or sheared DNA	incorrect storage of starting material	ensure the sample is harvested and stored at RT
	Old material	avoid repeated thawing and freezing cycles of the material. Old material often contains degraded DNA
problems with subsequent applications (e.g. in PCR)	ethanol in the eluted DNA	verify if the recommended dry time was reached increase dry time for the elimination of ethanol if necessary
	salt in the eluate	**Wash Buffer** should be stored at and used at RT verify **Wash Buffer** on the precipitation of salt. if there are precipitations dissolve this by carefully warming up to 30°C
	reduced sensitivity of amplification reaction	adjust the volume of eluate added as template in the amplification reaction
A_{260}/A_{280} ratio for purified DNA is low	insufficient cell lysis and protein degradation due to decreased **Proteinase K** activity	see above under " low amount of DNA"
	insufficient lysis due to insufficient mixing with lysis buffer	see above under " low amount of DNA"

Appendix

KingFisher BindIt Software 3.2 or higher versions

BindIt software 3.2 or higher versions were and may be used to create assay files for the KFmL, KF96/KFflex96 or KF-Duo instruments. The provided assay file(s) can either be transferred onto the corresponding workstation(s) or be started directly from within the BindIt software after assay import. Please keep in mind, that assay(s) run from within the BindIt software are not stored in the workstation memory.

Important: *Be advised that BindIt SW 3.2 or higher versions use a new unique file extension. Therefore, it is not possible to import assay files created with BindIt 3.2 or higher versions into older BindIt software versions! Please ask your local Thermo Scientific distributor for a software update.*

***Note**:* *When creating assay files for usage with KingFisher instruments in combination with Microtiter Deep Well plates (e.g. Thermo Electron), it is essential to use the KingFisher software 3.2 or higher versions for assay development because this software version includes the correct adjustments for the microtiter plate. It is highly recommended to use Thermo Microtiter Deep Well plates with KF96 / KFflex96 / KF-Duo workstations to ensure the best purification result.*

Minimum system requirements for BindIt Software 3.2 or higher versions

PC requirements	
Supported operating systems	MS Windows XP Pro with SP3, Windows Vista SP2, Windows 7
Disk space	500 MB free disk space
Processor	Intel Pentium ≥ 1 GHz
Memory	1 GB RAM
Serial ports available	1 (for KFmL connection)
USB ports available	1 (for KF96 / KFflex96 / KFDuo connection)
Pointing device	Mouse or equivalent is required
CD-ROM drive	1
Monitor / color settings	XVGA monitor with at least 1024x768 resolution and at least a 16-bit color environment

If the actual Windows Service Packs are not installed on the corresponding lab computer, they can be downloaded from the Microsoft web pages: http://www.microsoft.com/.

General notes on handling DNA

Nature of DNA
The length and delicate physical nature of DNA requires careful handling to avoid damage due to shearing and enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature, and UV irradiation. Careful isolation and handling of high molecular weight DNA is necessary to ensure compatibility with various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting, long-template PCR.

Storage of DNA
A working stock of DNA can be stored at 2-8˚C for several weeks. For long-term storage the DNA should be stored at -20˚C. However, storage at -20°C may cause shearing, particularly if the DNA is exposed to repeated freeze-thawing cycles.
Note that the used elution buffer will affect its stability during storage. Pure water lacks buffering capacity and an acidic pH may lead to acid hydrolysis. Tris or Tris-EDTA buffer contains sufficient buffering capacity to prevent acid hydrolysis.

Drying, dissolving and pipetting DNA
Avoid over-drying genomic DNA after ethanol precipitation. It is better to air dry than to use a vacuum, although vacuum drying can be used with caution.
Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings can cause shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for pipetting genomic DNA.

DNA yield
The amount of purified DNA depends on sample source, transport conditions, storage, and age of the sample.

Ordering information

Product	Package size	Catalogue No.
InviMag® SalivaGene DNA Kit/ KF96	1 x 96 preps	7435060100
InviMag® SalivaGene DNA Kit/ KF96	5 x 96 preps	7435060200
InviMag® SalivaGene DNA Kit/ KF96 w/o plastic	1 x 96 preps	7435060150
InviMag® SalivaGene DNA Kit/ KF96 w/o plastic	5 x 96 preps	7435060250
SalivaGene® Collection Module II	5 container	1035212100
SalivaGene® Collection Module II	50 container	1035212200
SalivaGene® Collection Module II	125 container	1035212300
SalivaGene® Buccal Swab	5 pieces	1035230100
SalivaGene® Buccal Swab	50 pieces	1035230200
SalivaGene® Buccal Swab	8 x 50 pieces	

Related products

InviMag® SalivaGene DNA Kit /KFmL	15 preps	2435160100
InviMag® SalivaGene DNA Kit /KFmL	75 preps	2435160200
InviMag® SalivaGene DNA Kit /KFmL	300 preps	2435160400
PSP® SalivaGene DNA Kit	50 preparations	1035200200
PSP® SalivaGene DNA Kit	250 preparations	1035200300

KingFisher 96 and consumables

KingFisher 96, Magnetic Particle Processor,100-240V,50/60Hz	5400500
KingFisher 96 Head for Deep Well plate	24073430
KingFisher 96 tip comb for PCR magnets, 8 x 10 pcs / box	97002514
KingFisher 96 tip comb for KF magnets, 10 x 10 pcs / box	97002524
KingFisher 96 tip comb for DW magnets 10 x 10 pcs / box	97002534
KingFisher 96 KF plate (200ul) 48 plates / box	97002540
Microtiter deep well 96 plate, 50 plates/box	95040450

Possible suppliers for Isopropanol:

Fa. Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Fa. Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

Fa. Sigma
2-Propanol
Order no. 59304-1L-F

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